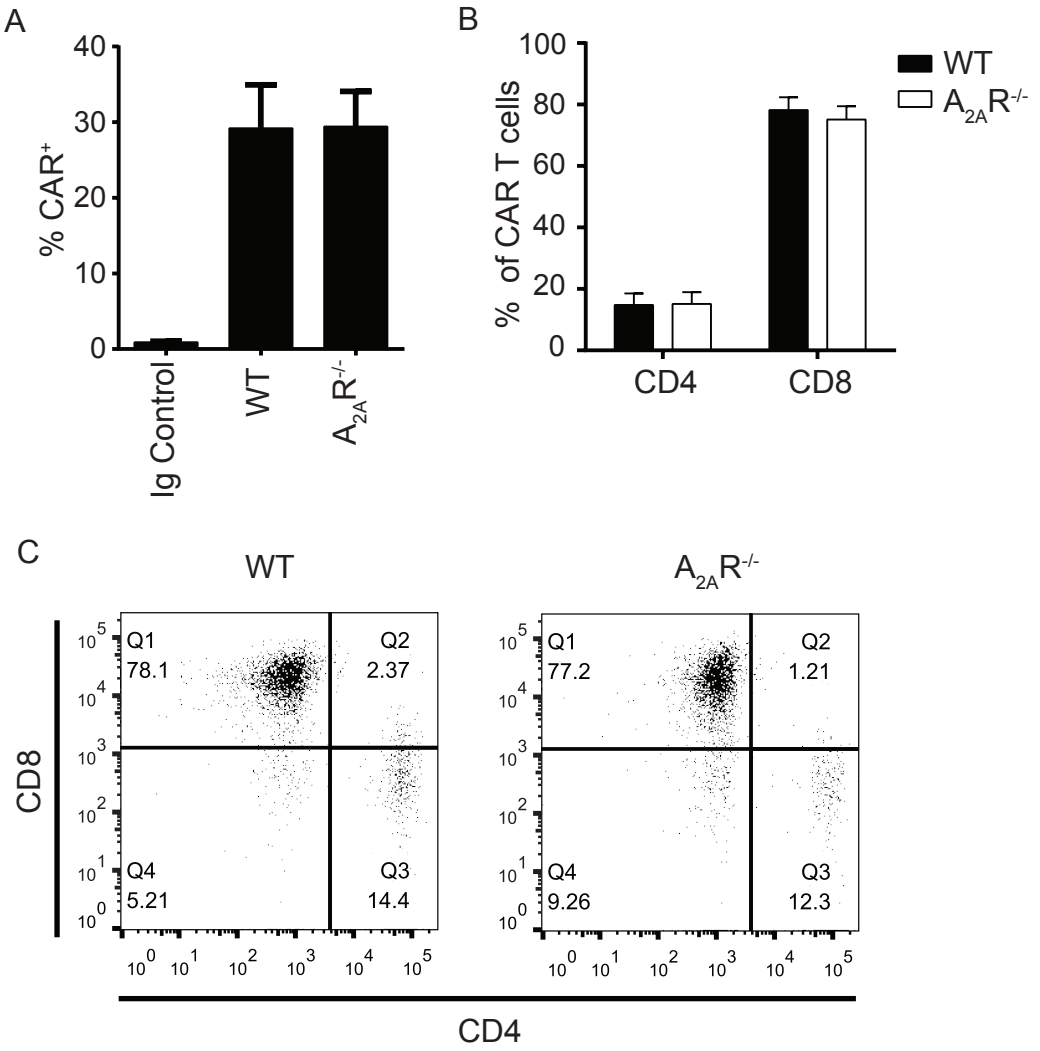
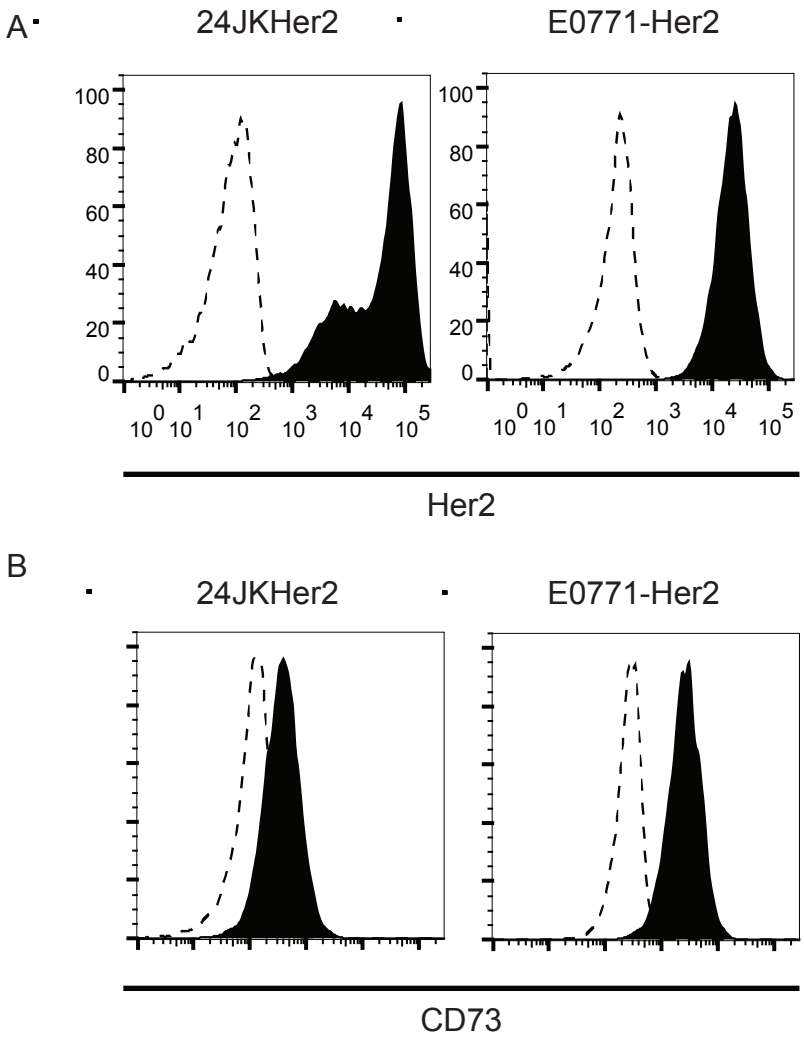
Supplementary Figure 1



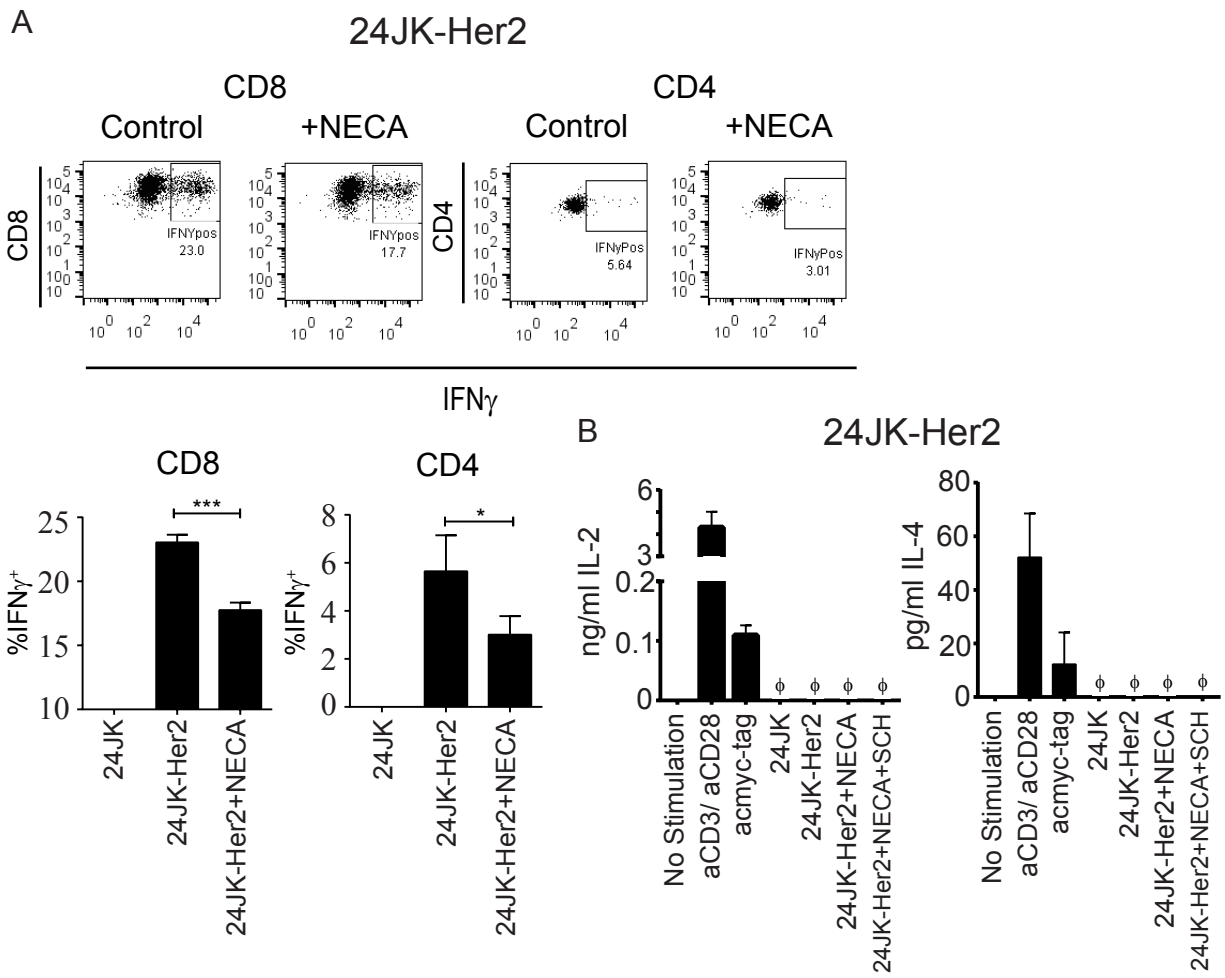
Supplementary Figure 1. Expression of the anti-Her2 CAR on WT and $A_{2A}R^{-/-}$ CAR T cells
Expression of the anti-Her2 CAR was determined by flow cytometry at day 7 post transduction. (**A)** Expression of the CAR on transduced T cells derived from WT and $A_{2A}R^{-/-}$ mice (**B-C)** Proportion of $CD4^+$ and $CD8^+$ T cells. (**A-B)** Data represented as the mean ± SEM of 8 individual experiments. (**C)** Data from a representative experiment.

Supplementary Figure 2



Supplementary Figure 2. Expression of Her2 and CD73 on murine tumor lines
The expression of Her2 (**A**) and CD73 (**B**) on 24JK-Her2 and E0771-Her2 was determined by flow cytometry. Dashed line indicates the staining with an isotype control antibody.

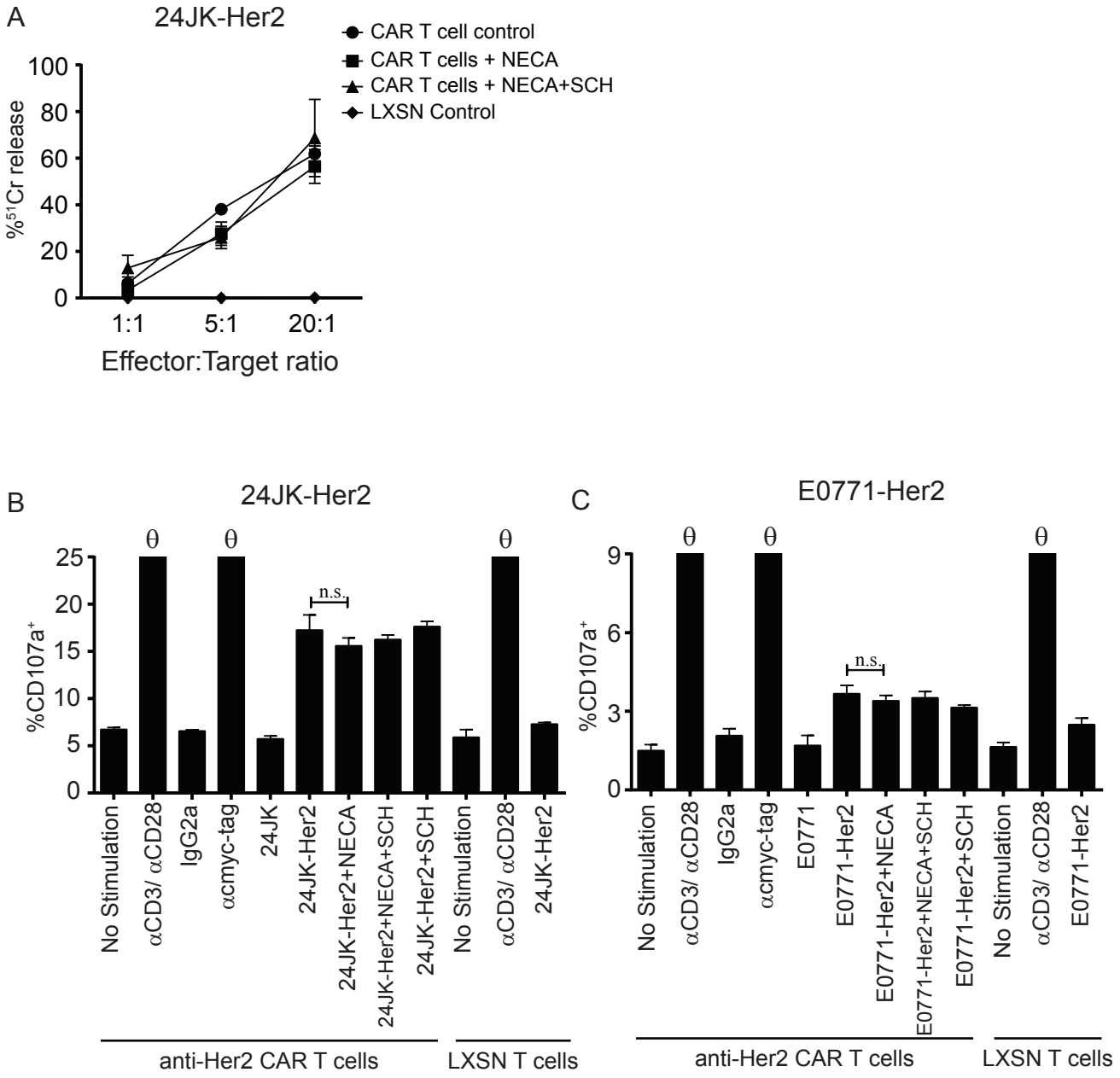
Supplementary Figure 3



Supplementary Figure 3. Cytokine production of anti-Her2 CAR T cells cocultured with 24JK-Her2 tumor cells.

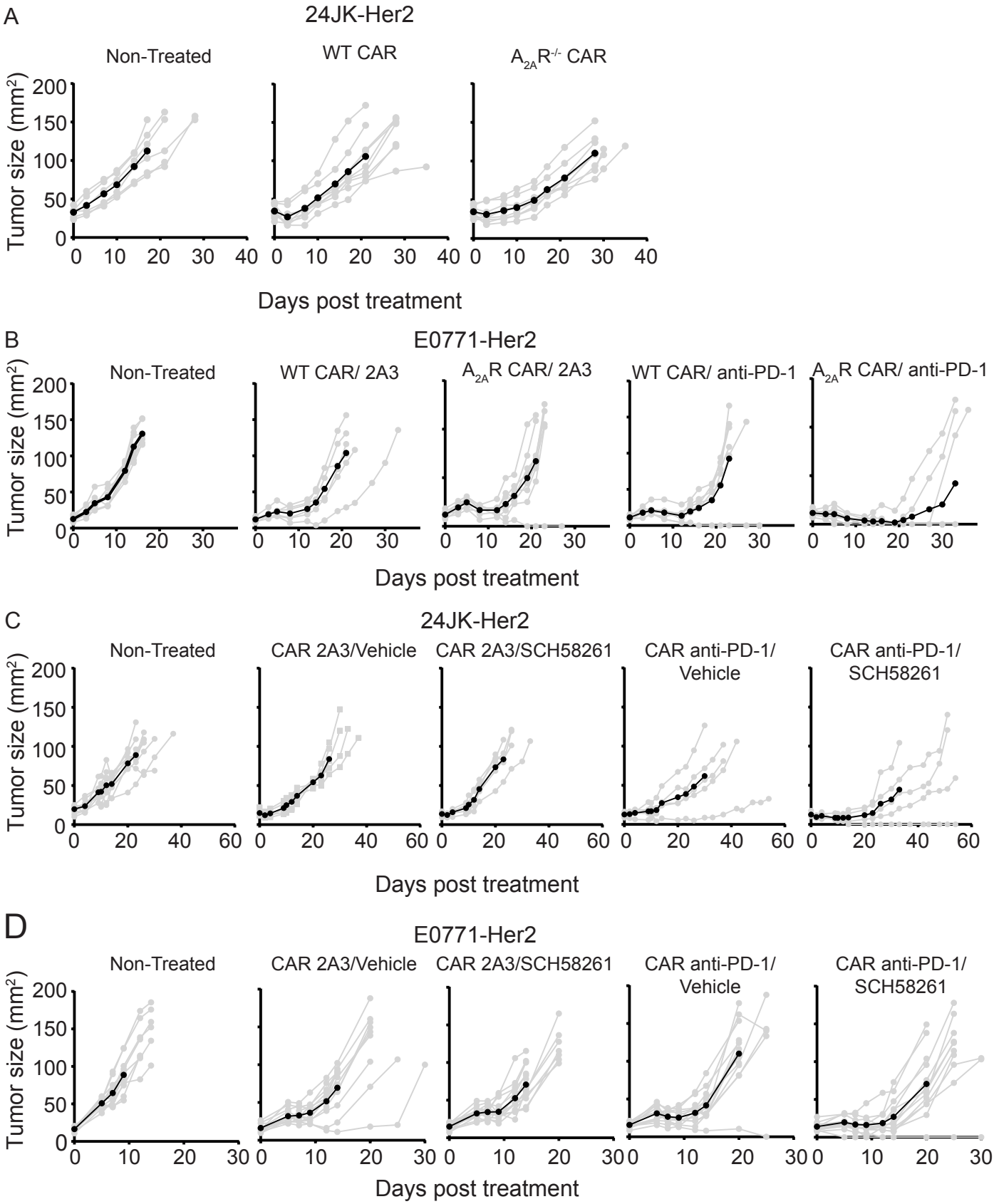
2 x 10^5 CAR T cells were cocultured with 1 x10^5 24JK/ 24JK-Her2 tumor cells in the presence or absence of NECA (1 µM) or SCH58261 (1 µM) . Following overnight coculture Golgi Plug and Golgi Stop were added for a further 4 hours of coculture and IFNγ production was determined by intracellular staining (**A)** or supernatants were harvested and the production of IL-2 and IL-4 determined by cytometric bead array (**B**). For a positive control, some anti-Her-2 CAR T cells were stimulated with αCD3/ αCD28 or α-cmyc-tag (αCAR stimulation). Results shown are the mean ± SD of triplicate cultures from a representative experiment of n= 3. ***p<0.001, *p<0.05 by paired t test, Φ indicates that level of cytokine was below the limit of detection.

Supplementary Figure 4



Supplementary Figure 4. $A_{2A}R$ activation does not suppress *in vitro* cytotoxicity of CAR T cells
(A) 1 x 10^4 24JK-Her2 tumor cells were labelled with ^{51}Cr and cocultured with the indicated number of CAR T cells for 4 hours. (**B-C**) 2 x10^5 CAR T cells were cocultured with 1 x10^5 24JK/ 24JK-Her2 tumor cells for 16 hours. Anti-CD107a, Golgi Plug and Golgi Stop were added for the last 4 hours of coculture. Cocultures were performed in the presence or absence of NECA (1 μM) and SCH58261 (1 μM). (**A-C)** Data is from a representative experiment of n= 4 and is shown as the mean ± SD of triplicates. LXSN T cells refers to T cells transduced with the empty retroviral vector (LXSN) control. n.s. not significant by one way ANOVA. θ- indicates that the recorded value was above the maximum indicated on the graph.

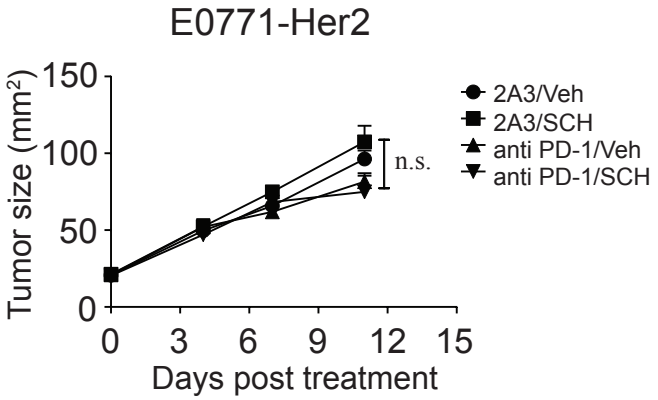
Supplementary Figure 5



Supplementary Figure 5. Combined targeting of $A_{2A}R$ and PD-1 leads to greater control of long term tumor growth by anti-Her2 CAR T cells

Long-term tumor growth is shown for mice injected with either 24JK-Her2 (**A,C**) or E0771-Her2 (**B,D**) and treated as per **Figure 2** (A,B) or **Figure 3 (C,D).** Individual long-term tumor growth curves are shown for each mice presented in **Figures 2** and **3 (**grey**)** with the mean tumor size of each group indicated by the black line.

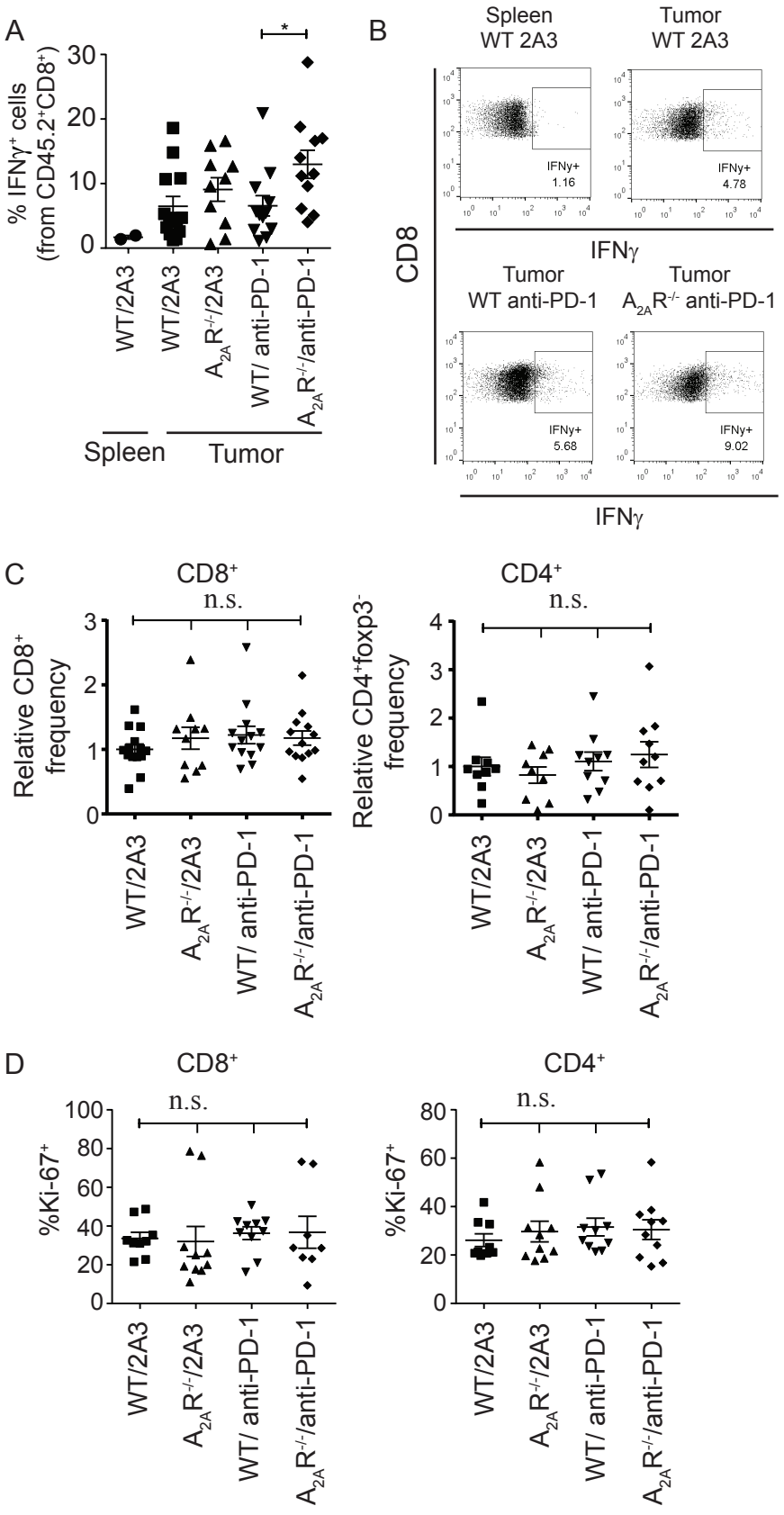
Supplementary Figure 6



Supplementary Figure 6. PD-1 and $A_{2A}R$ blockade is ineffective in controlling the growth of E0771-Her2 tumors in the absence of CAR T cells

C57/BL6-Her2 mice were injected with 1 x 10^5 E0771-Her2 tumor cells. Mice underwent total body irradiation (5 Gy) on day 7 and then treated with 2A3 isotype control or anti PD-1 (200 µg/ mouse) on days 0, 4 and 8 post irradiation. Mice were also treated daily with either 1 mg/ kg SCH58261 or vehicle control. Data is presented as the mean ± SEM of 6 mice from a representative experiment. n.s. not significant by two way ANOVA.

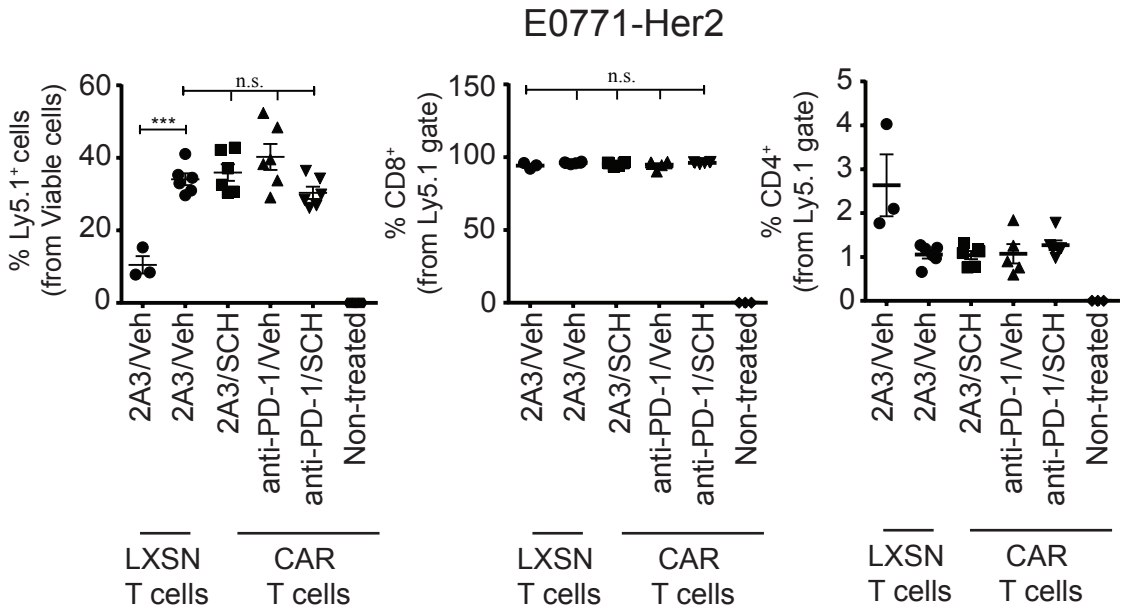
Supplementary Figure 7 E0771-Her2



Supplementary Figure 7. Phenotype of WT and $A_{2A}R^{-/-}$ tumor-infiltrating CAR T cells following PD-1 blockade

C57/BL6-Her2 mice were injected with 1 x 10^5 E0771-Her2 tumor cells and treated as per **Figure 3.** On Day 6 post treatment tumor-infiltrating lymphocytes were analysed by FACS. **(A-B)** Proportion of $CD3^+CD8^+$ cells that were $IFN\gamma^+$. (**A**) Data is shown as the mean ± SEM of at least 10 mice (**B**) Representative FACS plots. (**C**) Relative frequency of $CD3^+CD8^+$ and $CD3^+CD4^+$ T Cells from the viable $CD45^+$ gate**. (D)** Proportion of $CD8^+$ or $CD4^+$ cells expressing Ki-67. Data is shown as the mean ± SEM for at least 9 mice. *p<0.05, n.s. not significant by one way ANOVA.

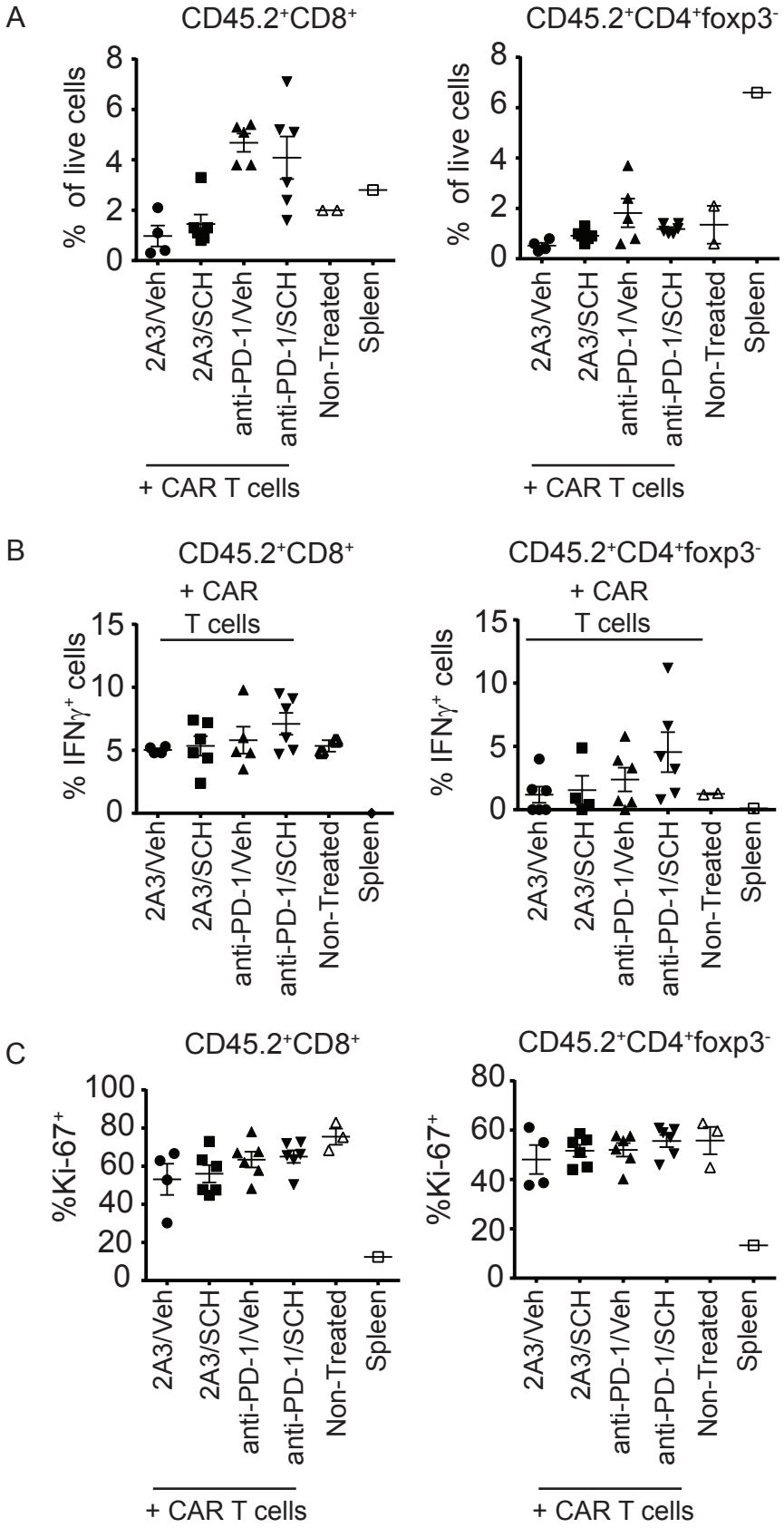
Supplementary Figure 8



Supplementary Figure 8. Phenotype of tumor-infiltrating CAR T cells following treatment with anti-Her 2 CAR T cells and PD-1/ A_{2A}R blockade

C57/BL6-Her2 mice were injected with 1 x 10^5 E0771-Her2 tumor cells. Mice were treated as per **Figure 3** using CAR T cells derived from Ly5.1$^+$ splenocytes**.** On Day 9 post treatment the frequency of Ly5.1$^+$, Ly5.1$^+$CD8$^+$ and Ly5.1$^+$CD4$^+$ cells was determined. Data is shown as the mean ± SEM for at least 3 mice from a representative experiment of n = 3. LXSN T cells refers to T cells transduced with the empty retroviral vector (LXSN) control. P<0.001, n.s. not significant by one way ANOVA.

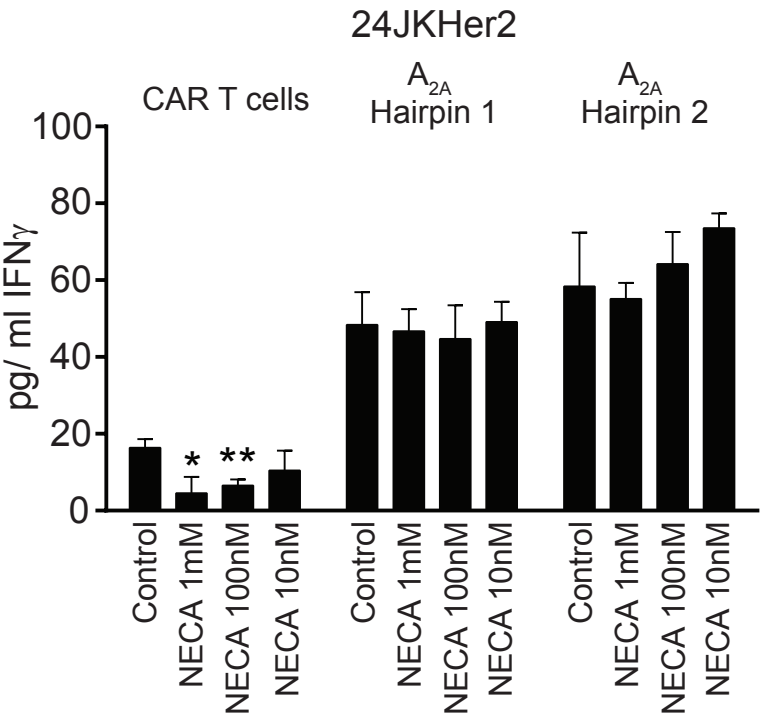
Supplementary Figure 9 E0771-Her2



Supplementary Figure 9. Phenotype of tumor-infiltrating endogenous T cells following treatment with anti-Her 2 CAR T cells and PD-1/ $A_{2A}R$ blockade

C57/BL6-Her2 mice were injected with 1 x 10^5 E0771-Her2 tumor cells. Mice were treated as per **Figure 3** using CAR T cells derived from $Ly5.1^+$ splenocytes**.** On Day 9 post treatment the frequency (**A**) IFNγ production (**B**) and expression of KI-67 (**C**) on tumor infiltrating endogenous $CD45.2^+CD8^+$ or $CD45.2^+CD4^+foxp3^-$ cells was determined. Data is shown as the mean ± SEM for at least 3 mice from a representative experiment. '+ CAR T cells' indicates analysis of endogenous T cells in mice treated with anti-HER2 CAR T cells and the indicated therapies.

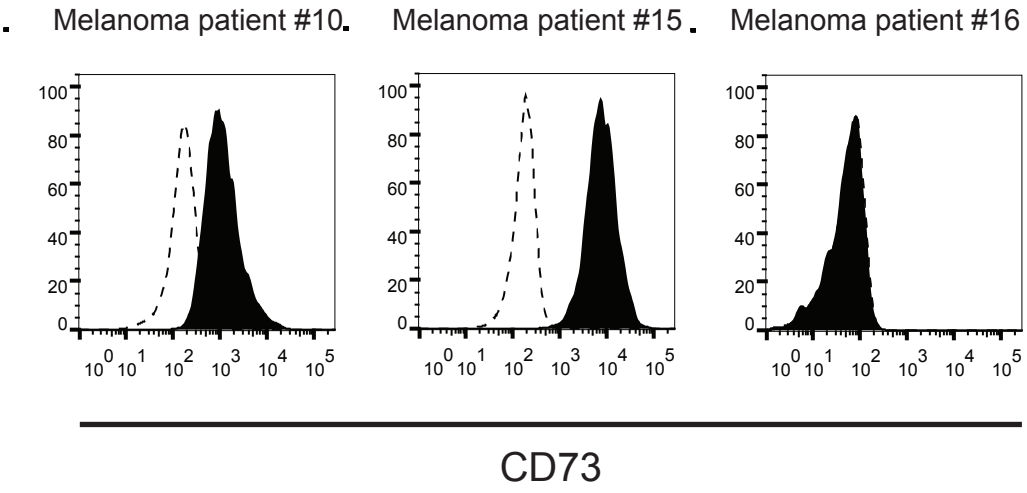
Supplementary Figure 10



Supplementary Figure 10. Targeting the $A_{2A}R$ with shRNA retroviral technology enhances CAR T cell IFNγ production

CAR T cells were transduced with retroviruses encoding $A_{2A}R$-directed shRNA or scrambled shRNA control and then selected with 2 μg/ ml puromycin from day 4-6. Eight days post activation CAR T cells were then assessed for function. $1x10^5$ CAR T cells were cocultured with 24JK/ 24JK-Her2 tumor cells at a 1:1 ratio in the presence or absence of NECA at indicated concentrations. Supernatants were collected after 16 hours and analysed for concentration of IFNγ. Data is the mean ± SD of quadruplicates from a representative experiment of n =2. * $p<0.05$, ** $p<0.01$ by one way ANOVA.

Supplementary Figure 11



Supplementary Figure 11. Expression of CD73 on patient derived melanomas.
The expression of CD73 on patient-derived melanomas was determined by flow cytometry. Dashed line indicates the staining with an isotype control antibody.